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A cis-acting element is necessary and sufficient for translational regulation of human ferritin expression in response to iron

(gene regulation/deletion mutagenesis)

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ABSTRACT **Ferritin plays a key role in determining the intracellular fate of iron and is highly regulated by the iron status of the cell. We have identified a cis-acting element in the transcribed but nontranslated 5′ leader sequence of human ferritin heavy-chain mRNA. In transiently transfected murine fibroblasts, the presence of a 157-nucleotide region of the 5′ leader sequence was found to be necessary for iron-dependent regulation of ferritin biosynthesis. Further, this 5′ leader region is sufficient to transfer iron-mediated translational control to the expression of a heterologous gene product, chloramphenicol acetyltransferase.**

Ferritin is the major intracellular repository of iron and exists as a heteropolymer of 24 subunits containing both heavy and light chains (1). It serves to sequester and thereby detoxify iron not otherwise utilized for cellular metabolism (2). A more active role of ferritin has been suggested (3), in which iron sequestration may limit and regulate iron availability for cellular metabolism. Ferritin's key role is highlighted by the following observations. (*i*) All eukaryotic cells investigated thus far have been shown to express ferritin (4). (*ii*) Cellular proliferation is dependent on the availability of iron, and ferritin levels are closely regulated during normal proliferation and have been found to be abnormal in various malignancies (5, 6). (*iii*) Ferritin in the cells lining the duodenal mucosa is likely to regulate intestinal iron absorption. Furthermore, since one of the most common autosomal recessive disorders, hereditary hemochromatosis, is characterized by pathologically increased absorption of dietary iron, abnormal regulation of ferritin expression or abnormal ferritin function could play a major role in this disorder (7). Thus, the regulation of ferritin levels by iron is the means by which ferritin can both influence and respond to iron homeostasis.

It is therefore important to elucidate the mechanisms by which ferritin gene expression is regulated. Munro and his colleagues (8, 9) suggested a translational regulation of ferritin expression in rat liver, based on two observations: (*i*) actinomycin D does not inhibit the stimulatory effect of iron on ferritin expression and (*ii*) cytoplasmic ferritin mRNA shifts from an inactive messenger-ribonucleoprotein pool to translationally active polysomes upon iron induction. Similar results were obtained for reticulocytes and liver of bullfrog tadpoles (10, 11). In further support of translational control of ferritin expression, cytoplasmic mRNA levels were shown to remain constant over a wide range of biosynthetic rates induced by iron (9). The recent cloning of the human ferritin heavy-chain cDNA and gene (12–15) has allowed us to directly address this question of the mechanism of biosynthetic regulation at a molecular level.

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Previous work showed that a 7.2-kilobase (kb) *Hin*dIII genomic DNA fragment (pUCM11) is actively transcribed in stably transformed and transiently transfected murine B6 fibroblasts (13), giving rise to a full-length human ferritin heavy-chain mRNA. The transformants express the human protein, which coassembles with the mouse heavy and light chains (16). Treatment of these cells with the iron chelator desferrioxamine or the iron source hemin results in the regulation of the biosynthetic rate of the human protein, and the consequent decrease or increase in ferritin protein expression is not accompanied by changes in total or cytoplasmic mRNA levels (16). We decided to investigate the molecular mechanism of this regulation by two approaches. Both focus on the 5′ nontranslated region of the ferritin mRNA, which is 212 nucleotides long and could influence the expression of ferritin at the level of translation initiation or ribosome scanning. The first approach was to test the effect of a major deletion from this region on the regulation of ferritin biosynthesis. The second approach was to attempt to transfer the proposed iron-dependent translational control to a heterologous gene product, chloramphenicol acetyltransferase (CAT). We report that the deletion of a major portion of the nontranslated 5′ leader sequence of the ferritin mRNA renders it unresponsive to iron. When the complete leader sequence is joined to the CAT gene, the translation of the resulting hybrid mRNA is controlled by iron.

MATERIALS AND METHODS

Construction of Ferritin-Related Recombinant Plasmids (see Fig. 1). The ferritin minigene (Fer-mg) is a full-length human ferritin heavy-chain cDNA under the control of its physiological promoter in the plasmid pUC18. It was generated by truncating pHF16, the cDNA clone isolated by Drysdale and colleagues (12), at its only *Sac* I site and ligating the resulting 3′ fragment of the cDNA to the 458-base-pair (bp) *Sac* I fragment of the genomic clone pUCM11 (13), which contains the 5′ promoter and leader sequences of the ferritin gene. This was followed by selection for the correct orientation and insertion into the *Sac* I (5′)–*Hin*dIII (3′) sites of pUC18. pFPBbv is a deletion construct from which nucleotides −30 to −186 of the ferritin leader sequence have been removed by truncation of the minigene and blunt-ending at a *Bbv* I site (−29). The resulting fragment was inserted into the *Hin*cII (5′)–*Hin*dIII (3′) sites of the ferritin promoter construct pFP_o (for details of construction of pFP_o, see *Results*). The two recombinant plasmids pFP_1-CAT and pFPL-CAT are both under the control of the ferritin promoter and express hybrid mRNAs coding for bacterial enzyme CAT. For the construction of pFPL-CAT, the 382-bp *Sac* I–*Tth*111.I fragment from the minigene was made blunt-ended at the *Tth*111.I site, isolated by gel electrophoresis, and inserted into the *Sac*

Abbreviation: CAT, chloramphenicol acetyltransferase.

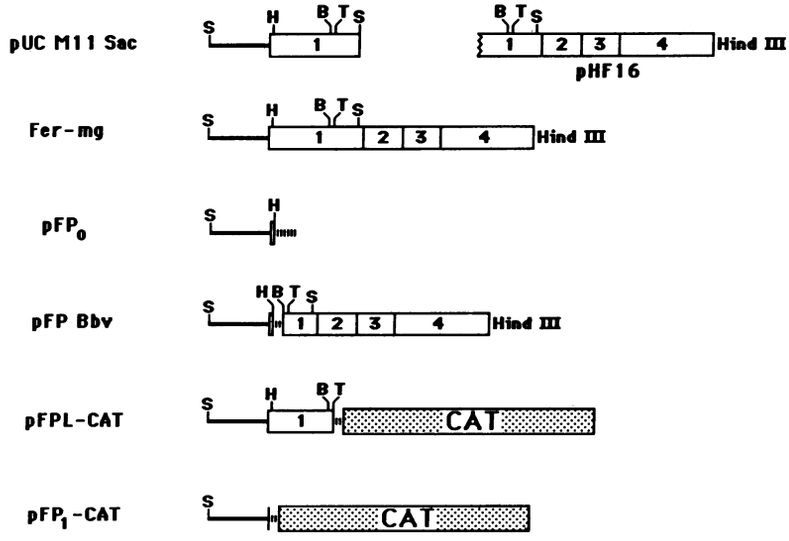

FIG. 1. Structure of recombinant ferritin constructs. Details of construction of these plasmids are in *Materials and Methods*. Solid lines represent the promoter and upstream region of the human heavy-chain gene, and open numbered boxes represent exons (13). Hash marks (▮) indicate pUC18 polylinker regions, and the stippled box represents the CAT GenBlock. Restriction enzyme sites: S, *Sac* I; H, *Hin*fI; B, *Bbv* I; T, *Tth*111.I. The translation start codon (ATG) of the ferritin heavy-chain cDNA is located in exon 1 between the *Bbv* I (B) and *Tth*111.I (T) restriction sites. pHF16, the human ferritin heavy-chain cDNA utilized in these constructs, lacks 120 5′ nucleotides of the complete cDNA, as indicated by the zigzag left-hand border of the bar representing pHF16.

I–*Sma* I sites of the pUC18 polylinker to form $pFPL_o$. Since the genuine ATG codon of the ferritin cDNA was included, the CAT GenBlock (Pharmacia) was inserted in-frame with its own ATG translation start codon by digesting $pFPL_o$ with *Xba* I, blunt-ending with the Klenow fragment of *E. coli* DNA polymerase, and inserting the blunt-ended CAT GenBlock in the correct orientation. The resulting hybrid mRNA contains the complete ferritin promoter and 5′ leader sequence and is expected to code for a CAT fusion protein with an N-terminal addition of 20 amino acids. The final construct was sequenced and the predicted structure was confirmed (Fig. 2). pFP_1-CAT was generated from pFPL-CAT by digestion with *Bam*HI and subsequent treatment with the exonuclease BAL-31. The reaction was stopped, the products were treated with DNA polymerase Klenow fragment, and the ferritin promoter fragment was released with *Sac* I and purified by gel electrophoresis. To determine the 3′ end of this promoter fragment accurately, we subcloned it into the *Sac* I–*Sma* I sites of the replicative form of bacteriophage vector M13 mp18 and determined its sequence by the dideoxy chain-termination method (17). It was then recovered from the M13 replicative form and inserted into the polylinker of pUC18. Finally, the blunt-ended CAT GenBlock was inserted 3′ into the *Hin*cII site and recombinant plasmids were selected for the correct orientation of the CAT structural gene with respect to the ferritin promoter fragment.

Transient Expression of Recombinant Plasmids. Murine B6 fibroblasts (10^6) were transfected in quadruplicate with 30 μg of plasmid DNA by use of the calcium phosphate precipitation method of Graham and van der Erb (18). After 16 hr, the precipitate was removed, and the cells were washed twice with medium and then incubated at 37°C in Dulbecco's modified Eagle's medium. For metabolic labeling and immunoprecipitation of ferritin, hemin (50 μM) or desferrioxamine (100 μM) were added to the medium 24 hr later, for 4 hr. Cells were biosynthetically labeled with a 2-hr pulse of [^{35}S]-methionine (Amersham) at 150 μCi/ml (1 Ci = 37 GBq). Immunoprecipitation of ferritin and analysis by $NaDodSO_4$/16.5% polyacrylamide gel electrophoresis were done as described (16). For determination of CAT activity, hemin (50 μM) or desferrioxamine (50 μM) were added to the medium 6 hr after removal of the calcium phosphate/DNA precipitate, and the cells were subsequently incubated for 22 hr at 37°C. Lysates were prepared, after two washes with ice-cold phosphate-buffered saline, by three freeze–thaw cycles in 0.25 M Tris/HCl buffer (pH 7.4) and subsequent heating to 56°C for 10 min. For each plasmid, equal aliquots of lysate were assayed from cells treated with hemin or desferrioxamine or from untreated cells and analyzed as described (16).

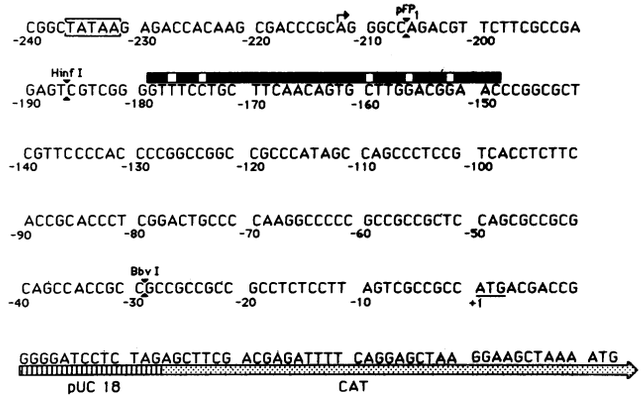

FIG. 2. Partial nucleotide sequence of pFPL-CAT. The nucleotide sequence of the human ferritin heavy-chain cDNA leader sequence and the joining region to the CAT structural gene is shown. The nucleotide sequence was determined by the dideoxy chain-termination method (17). The translation start site of the presumed fusion protein is numbered +1; the nucleotides of the 5′ noncoding region are given negative numbers. The suggested transcription start site (13) is marked with an arrow (position −212), and the "TATAA box" is bracketed. The 5′ boundaries of the constructs pFP_1-CAT and pFP_o and the 3′ boundary of the construct pFPBbv (see also Fig. 1 and *Materials and Methods*) are marked. The solid bar above the area −179 to −148 indicates the area of highest homology to the human ferritin light-chain leader sequence, with gaps indicating mismatches. The broad arrow marks the nucleotide sequence of the N-terminal addition to the predicted CAT fusion protein; sequence indicated by the hash-marked portion of the arrow originates from the pUC18 polylinker, and sequence indicated by the stippled portion represents the 5′ sequence of the CAT GenBlock.

Determination of Specific Hybrid CAT mRNA Levels in Murine Fibroblasts Transiently Transfected with pFPL-CAT. Cells (5.0×10^7) were transfected and treated as described above. Total RNA was prepared by solubilizing washed cells in guanidinium isothiocyanate and isolating RNA after ultracentrifugation through a 5.7 M CsCl cushion (19). Remaining DNA was removed by extensive treatment with RNase-free DNase I (Bethesda Research Laboratories). To generate a probe, the CAT GenBlock (Pharmacia) was blunt-ended and inserted into the *Hin*cII polylinker site of pFP_o (Fig. 1) to create pFP_o-CAT. A 439-bp *Eco*RI fragment spanning the ferritin promoter region and the 252 most 5′ nucleotides of the CAT GenBlock was excised and subcloned into pGEM-Blue (Promega Biotec). A single-stranded 450-bp "antisense" RNA probe was uniformly labeled with [α-^{32}P]GTP by *in vitro* transcription from the bacteriophage T7 promoter. This transcript (10^5 cpm) was hybridized to 50 μg of total RNA and then treated with 4000 units of nuclease S1 (Boehringer Mannheim) for 30 min at 40°C. The samples were phenol-extracted, ethanol-precipitated, resuspended, denatured, and analyzed by electrophoresis in an 8 M urea/6% polyacrylamide gel.

RESULTS AND DISCUSSION

Several experimental approaches to the question of translational control of ferritin expression were taken. We first tested the prediction that the complete cDNA under the control of its physiological promoter [a construct referred to as "ferritin minigene" (Fer-mg)] should exhibit iron regulation. To ensure that a full-length mRNA would be produced, the ferritin minigene was constructed as described above and schematically depicted in Fig. 1. Fig. 3 shows that the 161-bp promoter region upstream of the transcription start site is capable of directing the expression of the minigene in transiently transfected B6 cells. The band representing human ferritin migrates slightly above the lower of the bands of endogenous mouse ferritin. As predicted, the biosynthetic rate of human ferritin is iron-dependent, varying over a >100-fold range. This regulation is not accompanied by any changes in total or cytoplasmic ferritin mRNA levels (16). These iron manipulations do not lead to alterations in overall cellular protein biosynthesis as measured by total incorporation of [^{35}S]methionine into trichloroacetic acid-insoluble material (data not shown). Since ferritin mRNA levels are not affected by alterations in iron status, the promoter region of the ferritin minigene apparently allows transcription that is not influenced by iron. We therefore constructed an expression vector, pFP_o (Fig. 1), which was designed to include the physiological promoter and transcription start site and which thereby would allow predictable transcription of downstream nucleotide sequences. To create pFP_o, the 187-bp *Sac* I–*Hin*fI fragment of pUCM11 was isolated by gel electrophoresis, after blunt-ending at the *Hin*fI site, and inserted into the *Sac* I–*Sma* I sites of the polylinker of pUC18.

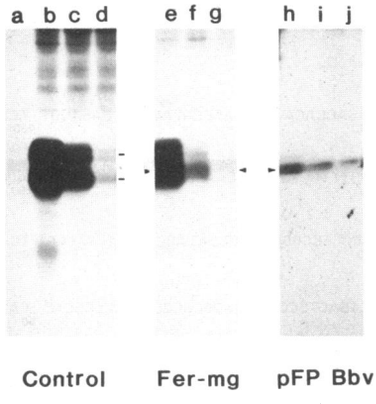

FIG. 3. The 5′ noncoding region of the human ferritin heavy-chain mRNA is necessary for translational regulation in response to iron. Murine B6 fibroblasts were transiently transfected with the ferritin minigene (Fer-mg, lanes e–g), the deletion construct pFPBbv (lanes h–j) or non-ferritin-related DNA [pSV2cat (20), lanes a–d]. Twenty-four hours after transfection, the cells were treated with 50 μM hemin (lanes b, e, and h) or 100 μM desferrioxamine (lanes d, g, and j) for 4 hr. Biosynthetic labeling, immunoprecipitation with saturating amounts of monoclonal anti-human ferritin heavy-chain antibody (lanes a and e–j) or polyclonal anti-ferritin antibody (lanes b–d), $NaDodSO_4$/polyacrylamide gel electrophoresis, and autoradiography were as described in *Materials and Methods*. The positions of endogenous mouse ferritin heavy and light chains (21.5 and 19.5 kDa, respectively) are indicated by markers to the right of lane d. Arrowheads next to lanes e, g, and h mark the position of the transfected human ferritin heavy-chain gene product, which migrates slightly above the lower mouse band. Due to the coassembly of human and murine ferritin subunits in the transfected B6 cells (16), the anti-human ferritin heavy-chain antibody can coprecipitate murine subunits (lanes e, f, and h). In contrast, when cells are transfected with pSV2cat, this antibody does not precipitate murine ferritin subunits in the absence of human ferritin (lane a).

Previous work has implicated the involvement of the 5′ leader sequences of the *Drosophila* heat shock gene *Hsp*70 (21), the yeast gene *GCN4* (22), and a β transcript of cytomegalovirus (23) in the translational regulation of these genes. We therefore utilized the pFP_o vector to test whether the deletion of a major internal portion of the 5′ leader sequence of the ferritin mRNA would affect the iron-mediated regulation of ferritin biosynthesis. The ferritin minigene was truncated and blunt-ended at a *Bbv* I site (−29, Fig. 1), removing all but the 29 most 3′ nucleotides from the leader sequence. This construct was inserted into the *Hin*cII–*Hin*dIII sites of pFP_o, creating an internal deletion of nucleotides −30 to −186 of the leader sequence bridged by the remaining *Sma* I–*Hin*cII polylinker sequences. The recombinant plasmid, pFPBbv, was transiently expressed in B6 cells. Fig. 3 shows that the resulting mRNA is translated into immunoprecipitable ferritin of normal size but has lost >95% of the biosynthetic responsiveness to perturbation of the cellular iron status. Scanning densitometry indicates a ratio of the biosynthetic rates after treatment with hemin or desferrioxamine of ≤2.6, as compared to the >100-fold range in the regulation of the complete ferritin minigene. Some of this apparent regulation of pFPBbv may be due to precipitation of coassembled and normally regulated endogenous mouse ferritin. These data prove that ferritin subunits can be synthesized and present within the cell yet be insensitive to the addition or removal of iron. We conclude that the iron regulation of the human ferritin heavy-chain biosynthesis is translational and that at least portions of the region between nucleotides −30 and −186 in the 5′ leader sequence are necessary to mediate this response.

To further evaluate the proposed role of the 5′ leader sequence, we asked whether the presence of this leader sequence would be sufficient to transfer the regulatory capacity to a heterologous mRNA. Fig. 2 shows a partial nucleotide sequence of pFPL-CAT, a plasmid constructed in order to address this question (see also *Materials and Methods*). pSV2cat (20), pFPL-CAT, and pFP_1-CAT were transiently expressed in B6 cells either in the absence of added agent or in the presence of hemin or desferrioxamine. pFP_1-CAT was generated by BAL-31 deletion from pFPL-CAT and retains only the six most 5′ nucleotides of the leader sequence. It served as a control for potential iron effects on the ferritin promoter. pSV2cat, in which the transcription of CAT mRNA is under the control of the simian virus 40 promoter/enhancer, functioned as a control for nonspecific effects of iron manipulation on the transfection or on the expression of CAT mRNA and/or enzyme. Fig. 4 shows the

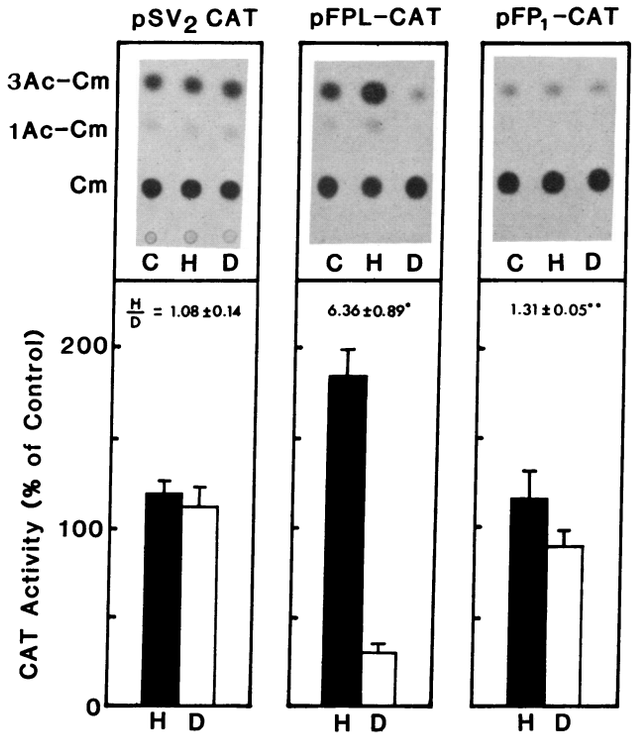

FIG. 4. The 5′ noncoding region of the human ferritin heavy-chain mRNA is sufficient to transfer iron-dependent regulation to a heterologous gene. pSV2cat (*Left*), pFPL-CAT (*Center*), and pFP_1-CAT (*Right*) were transiently expressed in mouse fibroblasts. Six hours after the transfection in quadruplicate, the cells were incubated with 50 μM hemin (H) or 50 μM desferrioxamine (D) for 22 hr or remained untreated (C). (*Upper*) Autoradiographs showing results of the CAT enzyme assay. 3Ac-Cm and 1Ac-Cm mark the position of the two monoacetylated products, and Cm marks the position of the unmodified [^{14}C]chloramphenicol substrate after thin-layer chromatography. (*Lower*) After autoradiography, the substrate and product spots were cut out and their radioactivity was determined in a liquid scintillation counter. The percentage conversion [(product cpm × 100)/(substrate cpm + product cpm)] was calculated and the results obtained with hemin (H, solid bars) or desferrioxamine (D, open bars) were expressed as relative percentages of the conversion rate of untreated cells. The range of the iron effect on the three constructs was determined by dividing the relative CAT activity under the influence of hemin by the relative CAT activity under the influence of desferrioxamine. The H/D ratios of pFPL-CAT and pFP_1-CAT, respectively, were compared with the H/D ratio of pSV2cat in a paired Student *t* test. For pFP_1-CAT (two asterisks) the *P* value was barely less than 0.05, indicating a possible minor effect of iron on the ferritin promoter. However, for pFPL-CAT (one asterisk) a *P* value of less than 0.001 was obtained in comparison with either pSV2cat or pFP_1-CAT, demonstrating the translational effect of iron on the expression of pFPL-CAT.

CAT enzyme assay results of these experiments. Iron perturbation has no effect on pSV2cat [hemin/desferrioxamine CAT expression ratio (H/D) = 1.08] and only a minor effect on pFP_1-CAT (H/D = 1.31). It is possible that this reflects a small transcriptional effect characteristic of the ferritin promoter. In contrast, the expression of pFPL-CAT is markedly affected by iron perturbation (H/D = 6.4). Hemin raises the relative CAT activity (expressed as percent conversion of the substrate) by a factor of 1.8, and desferrioxamine leads to a 3.6-fold decrease. The simultaneous addition of hemin and desferrioxamine abrogates the effect on the relative CAT activity of pFPL-CAT (data not shown). This finding demonstrates that the two agents can compete with each other and that they exhibit their effect via iron (24). It is important to note that the nature of the CAT assay only allows us to determine the total enzyme pool. Therefore, the regulatory effect of temporary iron perturbation on the biosynthetic rate of the enzyme is likely to be underestimated and cannot be directly compared with the range of regulation of the biosynthesis of ferritin. However, nucleotide sequences contained in the coding region or the 3′ untranslated region of the ferritin mRNA may influence the degree of regulation that is mediated via the cis-acting element of the 5′ leader region.

Because the iron effect on ferritin regulation does not involve a change in ferritin mRNA levels (9, 16) and because we utilized the ferritin promoter in pFPL-CAT, one would predict that the hybrid CAT mRNA levels in cells transfected with pFPL-CAT would be unchanged after hemin or desferrioxamine treatment. Fig. 5 demonstrates the validity of this prediction and demonstrates the translational nature of the iron effect on pFPL-CAT. A nuclease S1 protection analysis was performed on total RNA samples from transiently transfected B6 cells after DNase I pretreatment. We used a saturating amount of a uniformly ^{32}P-labeled RNA probe that was 450 nucleotides long (see *Materials and Methods*). Cells transfected with pFPL-CAT yield a specifically protected RNA, whereas control-transfected or B6 host cells give no specifically protected RNA. Hemin or desferrioxamine do not alter the quantity or size of the specifically protected RNAs when compared to untreated transfectants. Therefore the specific pFPL-CAT mRNA levels remain unchanged during iron perturbations, ruling out pretranslational regulation for the results shown in Fig. 4.

It could be argued that the addition of 10 nucleotides of ferritin coding region to pFPL-CAT or the addition of 20 amino acids to the N-terminus of the CAT protein could be responsible for the iron effect observed. To exclude this possibility, we analyzed a BAL-31 deletion construct that lacks the ferritin ATG translation start site but retains almost all of the 5′ leader region; the response of this construct to iron is identical to that of pFPL-CAT (data not shown). When the same BAL-31 deletion construct was linked to the gene coding for human growth hormone (hGH), the rate of

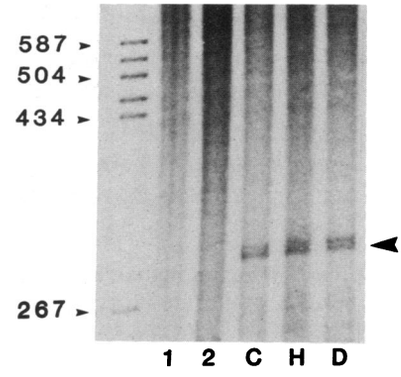

FIG. 5. pFPL-CAT mRNA levels remain constant during perturbations of cellular iron status in transiently transfected murine fibroblasts. Murine B6 fibroblasts were transiently transfected with pFPL-CAT (lanes C, H, and D) or stably transfected with non-CAT-related plasmid DNA (lane 2) or were left untreated (lane 1). Iron perturbations were performed exactly as described for Fig. 3. Total RNA was prepared (19) and treated with DNase I. Fifty micrograms of total RNA was hybridized to a 450-nucleotide-long "antisense" RNA probe (see *Materials and Methods*) and then treated with nuclease S1. The samples were analyzed by polyacrylamide gel electrophoresis followed by autoradiography. Arrowhead at right indicates the position of the specific pFPL-CAT-derived band, of a size predicted by protection of the CAT sequence, the polylinker sequence, and the transcribed portion of pFP_o. Size markers (lengths in nucleotides at left) were fragments generated by *Hae* III digestion of pBR322 (Boehringer Mannheim). The intensity of the band derived from cells treated with either hemin (lane H) or desferrioxamine (lane D) is unchanged when compared with the band derived from untreated cells (lane C).

secretion of hGH into the medium was regulated by iron perturbations in transiently transfected mouse fibroblasts. Since hGH secretion can more accurately reflect the biosynthetic rate of hGH, we were able to observe a much greater H/D ratio (≈30) than was seen with pFPL-CAT. Actinomycin D (5 μg/ml) had no effect on the iron-regulated expression of hGH encoded by these constructs. Thus transcription is not required for this regulation. We therefore conclude that the 5′ leader sequence of the ferritin heavy-chain mRNA is sufficient to transfer the translational regulation in response to iron to a heterologous mRNA.

Translational control of gene expression has been firmly established as an important mechanism by which prokaryotes regulate metabolic functions. More recently, elegant work on yeast, *Drosophila*, and cytomegalovirus has provided the first well-characterized examples from the eukaryotic kingdom. In all three cases the 5′ leader sequence was shown to mediate the regulated mRNA expression. In the case of the yeast gene *GCN4*, the main translation initiation codon is preceded by four small open reading frames that interact with trans-acting factors (22). Two small open reading frames are found within a region of the leader sequence of a cytomegalovirus β transcript that is necessary for the translational control of its expression (23). In the latter case, addition of the 5′ leader to an α (early)-gene construct converts the expression of the indicator protein to the β (or intermediate) class. The same area also contains a region of dyad symmetry that might allow a stem-and-loop structure to form. Kozak (25) has shown that experimentally introduced stem-and-loop structures can reduce the translational rate by a factor of 20. By applying the computer-modeling algorithm of Zuker and Stiegler (26), we have found that the ferritin leader sequence could allow a very high degree of intramolecular base-pairing (data not shown). Iron could influence the degree of secondary-structure formation through interaction with regulating molecules and thereby regulate the translational rate of ferritin. Biosynthesis of both the ferritin heavy and light chains is regulated by iron. Interestingly, a 31-nucleotide segment in the 5′ leader sequence of the ferritin heavy chain (−179 to −148, see Fig. 2) has 84% homology with a similarly located region in the 5′ leader sequence of the human ferritin light chain (27). The homologous regions could represent sites that are important for interaction with a common regulatory component. Additionally, the region from nucleotides −38 to −13 (Fig. 2) has up to 92% homology with a variable region of the human 28S rRNA (28). Whether or not this intriguing observation is of functional importance remains to be elucidated.

This report proves that the human gene for the ferritin heavy chain is regulated by translational control. We have identified the presence of a cis-acting element in the 5′ leader sequence of the mRNA coding for the heavy chain of human ferritin that is necessary for iron-dependent reversible translational regulation. We have localized this element to the region between 30 and 186 nucleotides upstream of the translation initiation site, and we have shown that the 5′ leader sequence itself is sufficient to transfer iron-dependent regulation to an indicator gene. Thomas and Thomas (29) have provided indirect evidence that other ubiquitous mammalian proteins (α- and β-tubulin, eukaryotic elongation factor 1α, poly(A)-binding protein, and vimentin) are under translational control during the early mitogenic response. Similar observations have been made for keratin (30). Understanding of translational control mechanisms in mammalian cells will provide deeper insights into molecular events underlying the regulation of gene expression.

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1. Aisen, P. & Listowsky, I. (1980) *Annu. Rev. Biochem.* **49,** 357–393.
2. Rice, D. W., Ford, G. C., White, J. L., Smith, J. M. A. & Harrison, P. M. (1983) in *Structure and Function of Iron Storage and Transport Proteins*, eds. Urushizaki, I., Aisen, P., Listowsky, I. & Drysdale, J. W. (Elsevier, Amsterdam) pp. 11–16.
3. Mattia, E., Josic, D., Ashwell, G., Klausner, R. D. & van Renswoude, J. (1986) *J. Biol. Chem.* **261,** 4587–4593.
4. Munro, H. N. & Linder, M. C. (1978) *Physiol. Rev.* **58,** 317–369.
5. Chou, C. C., Gatti, R. A., Fuller, M. L., Concannon, P., Wong, A., Chorda, S., Davis, R. C. & Salser, W. A. (1986) *Mol. Cell. Biol.* **6,** 566–574.
6. Hazard, J. T. & Drysdale, J. (1977) *Nature* **265,** 755–756.
7. Powell, L. W., Alpert, E., Isselbacher, K. J. & Drysdale, J. W. (1974) *Nature (London)* **250,** 333–335.
8. Zahringer, J., Baliga, B. S. & Munro, H. N. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 857–861.
9. Aziz, N. & Munro, H. N. (1986) *Nucleic Acids Res.* **14,** 915–927.
10. Shull, G. E. & Theil, E. C. (1982) *J. Biol. Chem.* **257,** 14187–14191.
11. Shull, G. E. & Theil, E. C. (1983) *J. Biol. Chem.* **258,** 7921–7923.
12. Boyd, D., Jain, S. K., Crampton, J., Barrett, K. J. & Drysdale, J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4751–4755.
13. Hentze, M. W., Keim, S., Papadopoulos, P., O'Brien, S., Modi, W., Drysdale, J., Leonard, W. J., Harford, J. B. & Klausner, R. D. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7226–7231.
14. Costanzo, F., Santoro, C., Colantuoni, V., Bensi, G., Raugei, G., Romano, V. & Cortese, R. (1984) *EMBO J.* **3,** 23–27.
15. Costanzo, F., Colombo, M., Staempfli, S., Santoro, C., Marone, M., Frank, R., Delius, H. & Cortese, R. (1986) *Nucleic Acids Res.* **14,** 721–736.
16. Rouault, T. A., Hentze, M. W., Dancis, A., Caughman, W., Harford, J. B. & Klausner, R. D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** in press.
17. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
18. Graham, F. L. & van der Erb, A. J. (1973) *Virology* **52,** 456–467.
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
20. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell Biol.* **2,** 1044–1051.
21. McGarry, T. J. & Lindquist, S. (1985) *Cell* **42,** 903–911.
22. Mueller, P. P. & Hinnebusch, A. G. (1986) *Cell* **45,** 201–207.
23. Geballe, A. P., Spaete, R. R. & Mocarski, E. S. (1986) *Cell* **46,** 865–872.
24. Rouault, T., Rao, K., Harford, J. B., Mattia, E. & Klausner, R. D. (1985) *J. Biol. Chem.* **260,** 14862–14867.
25. Kozak, M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2850–2854.
26. Zuker, M. & Stiegler, P. (1981) *Nucleic Acids Res.* **9,** 133–148.
27. Santoro, C., Marone, M., Ferrone, M., Costanzo, F., Colombo, M., Mingvanti, C., Cortese, R. & Silengo, L. (1986) *Nucleic Acids Res.* **14,** 2863–2876.
28. Laudien Gonzales, I., Gorski, J. L., Campen, T. J., Dorney, D. J., Erickson, J. M., Sylvester, J. E. & Schmickel, R. D. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7666–7670.
29. Thomas, G. & Thomas, G. (1986) *J. Cell Biol.* **103,** 2137–2144.
30. Tyner, A. L. & Fuchs, E. (1986) *J. Cell Biol.* **103,** 1945–1955.